Respiratory Medicine (2007) **101**, 2327–2335

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respiratoryMEDICINE

# The effect of smoking cessation and steroid treatment on emphysema in guinea pigs

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Received 7 November 2006; accepted 19 June 2007
Available online 9 August 2007

**KEYWORDS**
Adenovirus;
Cigarette;
Cytokine;
Emphysema;
Guinea pig

**Summary**
*Background:* Emphysema induced by cigarette smoking is characterized by an inflammatory process, which is resistant to steroid and remains active in lung tissue long after smoking has stopped. Latent adenoviral infection (Ad5) increases emphysema development and the inflammatory response to cigarette smoke and, in allergic lung inflammation, suppresses anti-inflammatory effects of steroids.
*Objectives:* The present study was designed to examine the effect of smoking cessation and steroid treatment on lung emphysema and inflammation in a guinea pig model of emphysema and to determine if latent adenoviral infection induces resistance to the inflammatory effects of steroid.
*Methods:* Latent adenovirus or sham infected animals exposed to room air or cigarette smoke for 16 weeks were either sacrificed immediately or treated with dexamethasone or diluent for an additional 5 weeks without smoke exposure. Lung morphometry, inflammatory cells and mediators were studied.
*Results:* Smoking cessation was associated with an increase in lung surface area and surface area to volume ratio. Smoking cessation was also associated with decreases in lung neutrophils, CD4 cells, and IL-8, RANTES and IFN-$\gamma$ mRNAs to control levels. Steroid treatment significantly lowered neutrophils, eosinophils and IFN-$\gamma$ mRNA and, while adenoviral infection did not alter these steroid-induced changes, it independently increased airway wall neutrophils and CD8 cells.
*Conclusion:* smoking cessation decreases lung inflammation and latent adenoviral infection does not induce steroid resistance in this animal model.


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doi:10.1016/j.rmed.2007.06.022

## Introduction

The cigarette smoking habit induces lung inflammation in everyone and this response is amplified in the 15–20% of smokers who develop chronic obstructive pulmonary disease (COPD). This inflammatory process[1,2] is characterized by an increase in neutrophils, macrophages and CD4 and CD8 T-lymphocytes.

While smoking cessation slows the rate of decline in $FEV_1$,[3] other studies[2,4,5] indicate that the inflammatory process remains active in lung tissue long after smoking has stopped. Steroid treatment has no effect on the rate of decline in lung function in COPD[6] but can prevent exacerbations and improve quality of life. No study has investigated the effect of steroid treatment on the inflammatory process in individuals who have stopped smoking, particularly with respect to the changes in lung histology associated with this dual intervention. Steroids decrease neutrophil apoptosis in vitro[7] and reports of short-term steroid treatment in patients with COPD suggest that steroids fail to reduce the number of neutrophils or their proteolytic activity in induced sputum from COPD patients.[8–10]

Chronic exposure of guinea pigs to cigarette smoke produces lesions similar to those seen in centrilobular emphysema in humans[11] and we found that latent adenoviral infection amplified both cigarette smoke-induced lung inflammation and the severity of emphysema.[12] Other studies from our laboratory[13] have also shown that latent adenoviral infection induces steroid resistance in allergen (ovalbumin)-induced lung inflammation in these animals.

The present study was designed to examine the effect of smoking cessation and steroid treatment on lung emphysema and inflammation in this guinea pig model of emphysema and to test the hypothesis that latent adenoviral infection induces resistance to the anti-inflammatory effects of steroid in this setting.

## Materials and methods

### Study design

This experiment was completed in two phases. Table 1 shows the way in which guinea pigs were randomized and the number of animals in each of the experimental groups. We exposed one group of sham (control) or infected animals (see below) to room air while another set of animals were exposed to cigarette smoke for 16 weeks followed by sacrifice (phase 1). A second set of latent adenoviral or sham infected animals received a similar 16 weeks of smoke exposure followed by 5 weeks in room air with or without steroid treatment prior to sacrifice and one group of sham (control) or infected animals received room air for all 21 weeks (phase 2, Table 1). The 5 weeks of room air exposure was to determine the effect of smoking cessation on inflammation induced by smoke exposure. Dexamethasone treatment during this smoking cessation period was to determine if steroids could reverse the smoke-induced inflammatory response and if latent adenovirus infection could induce steroid resistance The Committee on Animal Care of the University of British Columbia approved these studies.

### Animals

Female guinea pigs (Cam Hartley, 300–350 g) were purchased from Charles River (St. Constant, Quebec, Canada). Animals were provided with food and water *ad libitum* and their body weights were measured weekly throughout the experimental period.

### Adenoviral infection

Wild type adenovirus five was obtained from the American Type Culture Collection (Rockville, MD, USA). The virus was propagated in monolayer cultures of A549 cells and purified as previously described.[12] For sham infections, non-infected A549 cells were cultured and processed as the infected cells.

Animals were anesthetized with Halothane® and then given either $10^{9.2}$ $ID_{50}$ of purified adenovirus or the equivalent volume of the sham infection preparation in 200 μl PBS intranasally. The infected animals were kept in containment for 21 days to allow latent infection to be established.[12] The presence of latent adenoviral infection was confirmed by $TCID_{50}$ assay of the lung homogenates and by in situ hybridization of paraffin-embedded lung sections from three adenovirus infected animals 7 days post-infection while a corresponding sham infected animal served as a negative control (data not shown).

### Cigarette smoke exposure

After 21 days, awake animals were exposed to non-filtered cigarette smoke or room air using a previously described method and apparatus.[12] These smoke and room air exposures took place over a 16-week period. At the end of 16 weeks, phase 1 animals were sacrificed.

### Steroid treatment

At the end of 16 weeks, smoke exposure was stopped in phase 2 animals. Some of these animals were treated during the next 5 weeks with a daily oral dose of 10 mg/kg of dexamethasone $C_{22}H_{29}FO_5$ (Ultrapure from USB) diluted in 5% D-glucose solution or received an equal volume of the vehicle delivered in the same way while animals not exposed to cigarette smoke received neither (Table 1). The dose and the route of administration of the dexamethasone were based on preliminary work done in our laboratory.

### Lung tissue preparations

All animals were coded then euthanized with an intraperitoneal overdose of pentobarbital. After sacrifice, animals in the first phase that were exposed to cigarette smoke for 16 weeks had their lungs removed and their right upper and lower lobes inflated and fixed in 10% buffered formalin and lung volume measured by water displacement normalized to body weight to serve as a reference volume for morphometric analysis. These two lobes were processed into paraffin blocks to provide the histological sections required for morphometric and immunohistochemical analysis.[12] The

**Table 1** Study design and number of animals.

| *Study* | | | | | | |
|---|---|---|---|---|---|---|
| Phase 1 | | | | | | |
| | | Week 1–16 | | | | |
| Sham | → | Air (control) | → | Sacrifice (5) | | |
| | | Smoke | → | Sacrifice (5) | | |
| Ad | → | Air | → | Sacrifice (5) | | |
| Phase 2 | | | | | | |
| | | Week 1–16 | stop | Week 17–21(Air) | | |
| Sham | → | Air (control) | → | No treatment | → | Sacrifice (8) |
| | | Smoke | → | Vehicle | → | Sacrifice (10) |
| | | Smoke | → | Dex | → | Sacrifice (10) |
| Ad | → | Air | → | No treatment | → | Sacrifice (8) |
| | | Smoke | → | Vehicle | → | Sacrifice (9) |
| | | Smoke | → | Dex | → | Sacrifice (9) |

Phase 1: Sham-infected guinea pigs were exposed to cigarette smoke for 16 weeks or room air and then sacrificed. Phase 2: Guinea pigs were Ad or sham-infected and exposed to cigarette smoke or room air for 16 weeks followed by 5 weeks at room air before they were sacrificed. Ad or sham-infected animals exposed to cigarette smoke were randomized to received Dex or vehicle during the 5 weeks of room air exposure while Ad or sham-infected 16 week air control received neither. Dex: dexamethasone, Ad: Latent adenovirus infection, (*n*).

right middle lobe was frozen in liquid nitrogen and stored at $-70\,^{\circ}C$ for RNA extraction. The left lung was inflated in a 1:1 mixture of PBS (pH 7.4) and CryoMatrix (Tissue-Tek, Miles Inc., Elkhart, IN, USA), snap frozen in liquid nitrogen then stored at $-70\,^{\circ}C$ for use in immunohistochemical analysis. Lung tissues from the animals in phase 2 were processed in a similar manner except for the following differences. The right upper and middle lobes were directly processed for RNA extraction; the right lower lobe processed as above for immunohistochemical analysis of frozen sections while the left lung inflated with 10% buffered formalin for paraffin embedding.

## Lung morphometry

To determine the alveolar surface area (SA), lung SA/volume ratio and volume fractions of lung compartments (alveolar tissue, airspace and airway wall) sections cut from the paraffin blocks onto glass slides were stained with hematoxylin and eosin. Whole mount scans of glass slides were used first for dissecting the parenchymal from the non-parenchymal tissue such as large conducting airways and blood vessels. In order to provide a coefficient of error $<0.1$, at least five fields per slide were randomly selected. All the fields were captured using a $10\times$ objective by the Cool Spot digital camera and images were analyzed using ImagePro Plus software. A multilevel cascade design[14] was used to determine the volume fractions ($V_v$) of lung tissue, airspace, airway wall, airway lumen and lung surface density ($S_v$). The total SA of lung parenchyma was calculated by multiplying $S_v$ by the total lung volume.

## Immunohistochemical analyses

T-lymphocyte subsets and macrophages were identified by immunostaining of frozen serial sections fixed in 10% formalin.[12] Neutrophils and eosinophils were identified on subsequent serial sections stained with diaminobenzidine and Hansel's method, respectively. The accumulated volume of specifically stained cells located in the parenchyma or airway wall was determined by point counting images captured using light microscopy as previously described.[12] By applying the multilevel cascade design this volume was expressed as total stained cell volume (ml) per kg of body mass.

## RNA extraction

Approximately 2 g of frozen inflated lung tissue from phase 1 animals sacrificed after smoke exposure were homogenized in 15 ml of lysis buffer supplied by the RNeasy Maxi Kit (Qiagen, Mississauga, Ont., Canada). The homogenate was frozen in liquid nitrogen and stored at $-70\,^{\circ}C$ until RNA was extracted. Similarly RNA was extracted from approximately 0.6 g of non-inflated tissue from the right upper and middle lobes of phase 2 animals.

## Real-time PCR of IL-8 and RANTES and semi-quantitative PCR of IFN-$\gamma$ mRNA

One $\mu$g of total RNA, previously treated with DNAse, was reverse transcribed using the murine Moloney leukemia virus reverse transcriptase and random hexamers. PCR for IL-8, RANTES, IFN-$\gamma$, and 18S used primers listed in Table 2, SYBR Green PCR core reagents and a real-time PCR Applied Biosystems Prism 7600 sequence detector (Applied Biosystems, Foster City, CA, USA) following manufacturer's instructions. The concentration for IL-8, RANTES and 18S rRNA in the samples was determined using standard curves from their respective cDNA. IL-8 and RANTES mRNA concentrations were normalized to that of the corresponding 18S rRNA. Because the expression of IFN-$\gamma$ in these

**Table 2** PCR primers used to amplify inflammatory mediator mRNAs.

| Mediator | Sequences | Reference |
|---|---|---|
| IL-8 | | 15 |
| Forward | 5′-GGCAGCCTTCCTGCTCTCT-3′ | |
| Reverse | 5′-CAGCTCCGAGACCAACTTTGT-3′ | |
| RANTES | | 16 |
| Forward | 5′-TGCTGCTGCTTGTTCTGGTCTT-3′ | |
| Reverse | 5′-GCAGGGCCTCGATAGCTCTTT-3′ | |
| IFN-$\gamma$ | | Gene Bank: gi13024975 |
| Forward | 5′-TCAGAGCCAAATCGTCTCCTTC-3′ | |
| Reverse | 5′-GTTCGGTGACAGGTCATCTATC-3′ | |
| 18S rRNA | | 15 |
| Forward | 5′-TGCATGGCCGTTCTTAGTTG-3′ | |
| Reverse | 5′-AGTTAGCATGCCAGAGTCTCGTT-3′ | |

samples was low and could not be quantified reliably by real-time PCR, PCR products amplified after 35 cycles were, therefore, detected on ethidium bromide stained agarose gels and the band representing the IFN-$\gamma$ product was analyzed semi-quantitatively with the NIH Imaging Software. The density of the IFN-$\gamma$ bands was compared to IFN-$\gamma$ cDNA standards run on the same gel before normalizing to the corresponding 18S rRNA from above.

### Statistical analysis

A nested model of analysis of variance was used to assess the relevant effects of smoking cessation, steroid treatment and latent adenoviral infection on lung volume, SA, SA to volume ratio and the volumes of the inflammatory cells; each main effect and interaction was tested using a contrast.[17] The analyses of IL-8, RANTES, IFN-$\gamma$, were performed using two-way ANOVA. The normality assumption was verified with the Shapiro–Wilk test and the Brown and Forsythe's variation of Levene's test statistic was used to verify the homogeneity of variances. After performing these tests on raw data, all $p$-values were $<0.05$, meaning that these assumptions were not fulfilled and were rejected. The graphical analyses of residuals with predicted values revealed a relationship between the variances of the observations and the means for these variables. To estimate the form of the required transformation associated to these variables, a regression approach was performed between the logarithm of the standard deviations and the logarithm of the means from different conditions. This approach was useful to stabilize the variance.[17] Some of the parameters were log transformed (RANTES/18S) and an inverse transformation (IL-8/18S, IFN-$\gamma$/18S) was applied to the others. As normality assumptions were not obtained for some transformed parameters, a generalized linear model using a gamma distribution with an inverse link function was used. Results from these statistical models were similar to ones obtained from the ANOVA on transformed data. Statistical results from these parameters were expressed with transformed values. The Tukey's multiple comparison technique was applied *post hoc* to the ANOVA. The results were considered significant with $p$-values $\leqslant 0.05$. The data were analyzed using the statistical package program SAS v8.2 (SAS Institute Inc., Cary, NC, USA).

## Results

Weekly recordings of body weights during the smoke exposure periods showed similar overall weight gain in the control and treated animals in both phase 1 and 2 studies (data not shown). Cortisol levels measured at the end of study were lower in the dexamethasone-treated animals versus vehicle-treated ones (1045 nmol/l, $n = 19$ versus 1648 nmol/l, $n = 19$; $p = <0.00001$). Although steroid treatment resulted in a slower rate of weight gain, the final weights were not different between any of the groups ($p > 0.05$).

Because of the variability of the control groups between the two separate sets of experiments (smoking and smoking cessation protocol) we decided to express the results relative to their respective controls. Furthermore, when we compared the normalized values to those expressed as absolute values we found that the conclusions reached remain unchanged whether the data was expressed as ratios or as absolute values (data not presented).

### Emphysematous changes

Animals exposed to smoke until sacrifice and those where smoking was stopped 5 weeks prior to sacrifice were compared to their respective sham room air controls (Table 1). Smoke exposure increased lung volume (Figure 1A) and decreased lung SA/volume ratio (Figure 1B) but did not change lung SA (Figure 1C). When the same level of smoke exposure was followed by 5 weeks of smoking cessation, lung volumes remained high (Figure 1A) but the lung SA/volume ratio increased (Figure 1B). Surprisingly, there was a significant increase in lung SA (Figure 1C) following smoking

cessation with no additional effect with either latent adenoviral infection or steroid treatment.

## Parenchyma inflammation

Inflammatory responses in the lung parenchyma of animals from the two phases compared to their respective controls (Figure 2) showed the following. Parenchymal neutrophils were increased after the smoke exposure and smoking cessation lowered neutrophil counts to control levels independently of latent adenoviral infection (Figure 2A). The administration of steroid during the smoke cessation period had an independent effect in lowering these neutrophil counts in the parenchyma below control levels and this was not influenced by latent adenoviral infection (Figure 2A). Smoke exposure increased parenchymal macrophages, stopping smoke did not fully reverse this while adenovirus or steroid had no additional effect (Figure 2B). The analysis of the inflammatory response showed that following smoke exposure and cessation, both the parenchymal CD4 and CD8 T-lymphocytes were not statistically different between the groups (data not shown).

## Airway inflammation

Analysis of the airway wall showed that smoke exposure increased neutrophils while smoking cessation allowed their numbers to return to control values (Figure 3A). Latent adenoviral infection also caused an increase in neutrophils despite smoking cessation. Steroid treatment independently lowered their counts below control levels (Figure 3A).

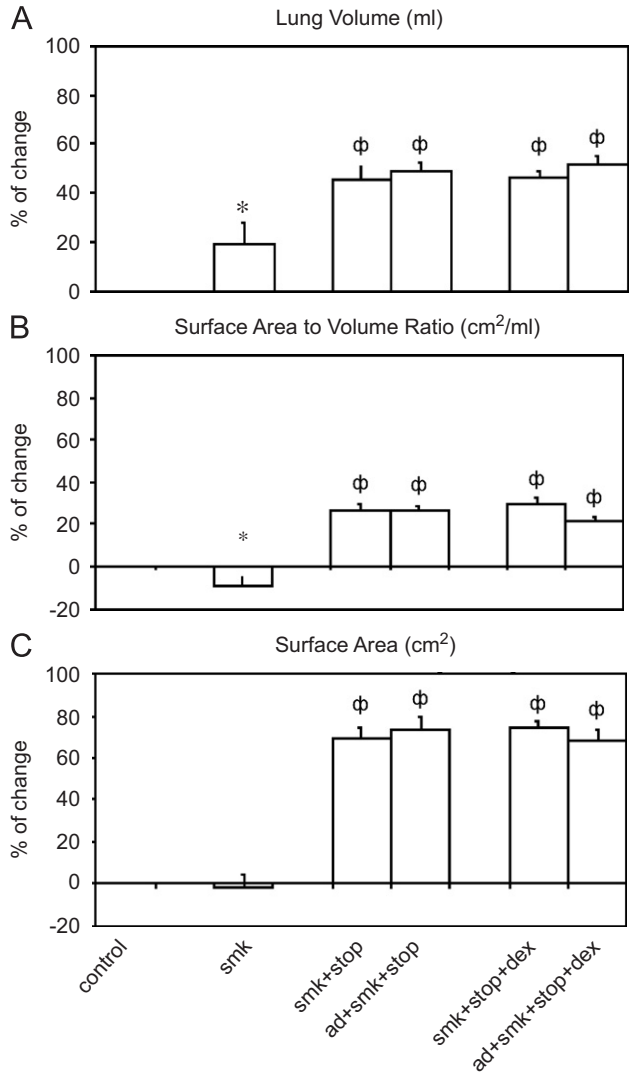


**Figure 1** The independent and combined effects of smoke exposure (smk), smoke cessation (smk+stop), latent adenoviral (ad) infection and steroid treatment (dex) on total lung volume (A), lung surface area to volume ratio (B) and surface area (C) of the lung. Each treatment group is normalized to its respective sham infected, room air control (control) where the values were $22.7 \pm 3.25$ and $23.9 \pm 0.74$ ml (A), $437 \pm 9.54$ and $613 \pm 14.8\,cm^2/ml$ (B), and $10{,}800 \pm 415.8$ and $14{,}595 \pm 452.5\,cm^2$ (C) for phase 1 and 2 controls, respectively. Data are presented as a % of change compared to control $\pm$ S.E.M. *Main effect of smoke exposure (A, $p = 0.04$) and (B, $p = 0.001$); ΦMain effect of stopping smoking (A, $p = 0.0001$), (B, $p = 0.0001$) and (C, $p = 0.0001$).

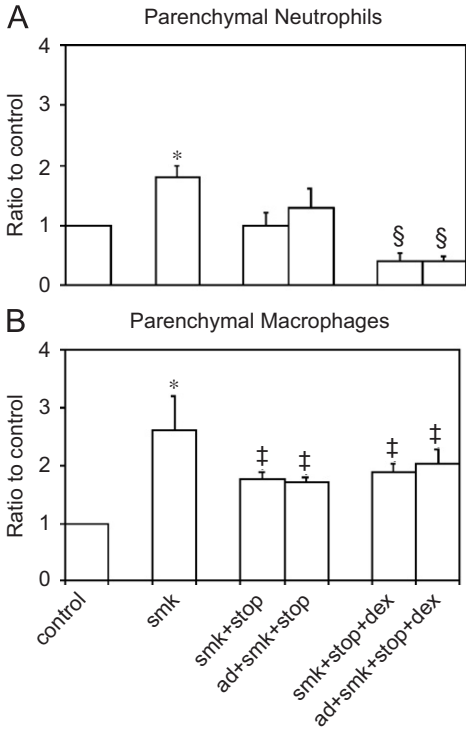


**Figure 2** The response of parenchymal neutrophils (A) and macrophages (B) to smoke exposure, smoke cessation, latent Ad infection and steroid treatment. The designation of the treatment groups and controls are the same as in Figure 1. Absolute volumes of each cell type were determined by the multilevel sampling design and analyzed using ANOVA. Each treatment group is normalized to its respective control where the volumes of neutrophils are $0.103 \pm 0.05$ and $0.189 \pm 0.03$ ml/kg (A), and the volumes of macrophages are $0.071 \pm 0.02$ and $0.391 \pm 0.04$ ml/kg (B) for phase 1 and 2 controls, respectively. *Main effect of smoke exposure (A, $p = 0.0001$), (B, $p = 0.001$); ‡(B, $p = 0.0004$ compared to control); §Main effect of steroid treatment (A, $p = 0.0005$).

Airway wall eosinophils were markedly reduced by steroid treatment but neither latent adenovirus, smoke exposure, nor stopping smoking had any effect (Figure 3B). CD4 lymphocytes in the airway were only affected by cigarette smoke which led to their increase while smoking cessation allowed them to return to control values (Figure 3C). CD8 T-cells, in contrast, were only affected by latent adenoviral infection, which independently led to their increase (Figure 3C).

### Inflammatory mediator expression

IL-8 expression increased only in uninfected animals after smoke exposure (Figure 4A). IL-8 expression was not affected by latent adenovirus alone (data not shown) or by latent adenovirus infection or steroid treatment after stopping smoking but smoking cessation returned it to control levels (Figure 4A). Cigarette smoke exposure increased RANTES mRNA levels in the guinea pig lungs (Figure 4B) and its expression returned to control levels after 5 weeks of smoking cessation. Neither latent adenoviral infection alone (data not shown) or in combination with smoking cessation nor steroid treatment altered RANTES expression (Figure 4B). IFN-$\gamma$ mRNA was also increased by smoke exposure (Figure 4C) and returned to respective control levels after smoking cessation (Figure 4C). Latent infection alone did not have any effect (data not shown). Steroid treatment significantly decreased IFN-$\gamma$ mRNA compared to control while latent adenovirus infection had no additional effect (Figure 4C).

## Discussion

The results of these animal studies demonstrate that smoking cessation reduced lung inflammation and increased lung SA suggesting that tissue repair processes were

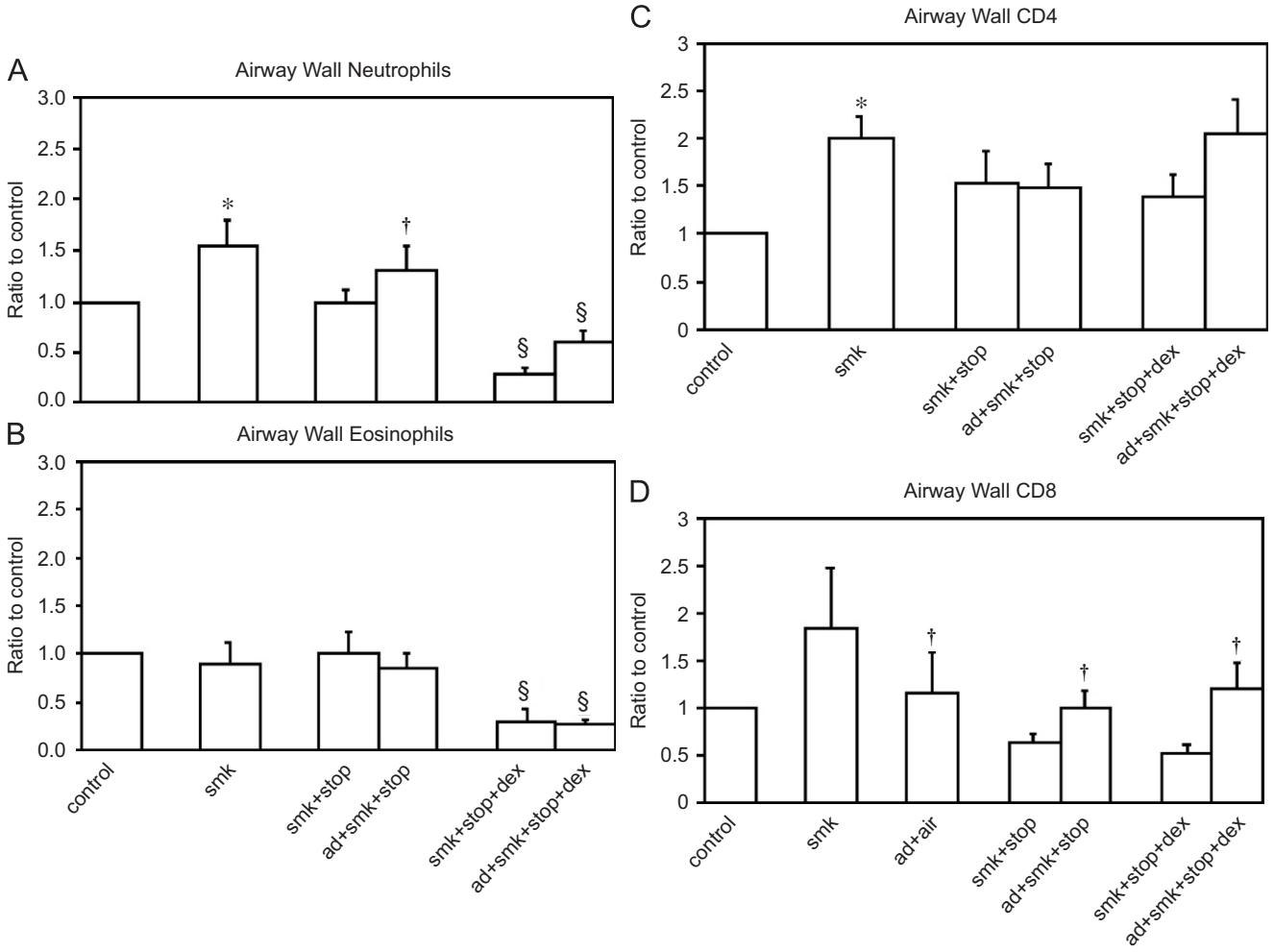


**Figure 3** The response of airway wall neutrophils (A), eosinophils (B), CD4 (C) and CD8 (D) T-cells to smoke exposure, smoke cessation, latent Ad infection and steroid treatment. The designation of the treatment groups and controls are the same as in Figure 1. Absolute volumes of each cell type were determined by the multilevel sampling design and analyzed using ANOVA. Each treatment group is normalized to its respective control where the volumes of neutrophils are $0.021 \pm 0.001$ and $0.051 \pm 0.004$ ml/kg (A), the volumes of eosinophils are $0.043 \pm 0.007$ and $0.021 \pm 0.004$ ml/kg (B), the volumes of CD4 are $0.02 \pm 0.003$ and $0.108 \pm 0.024$ ml/kg (C) and the volumes of CD8 are $0.009 \pm 0.0008$ and $0.055 \pm 0.0005$ ml/kg (D) for phase 1 and 2 controls, respectively. *Main effect of smoke exposure (A, $p = 0.0002$) and (C, $p = 0.001$); †Main effect of latent Ad infection (A, $p = 0.005$), and (D, $p = 0.007$); §Main effect of steroid treatment (A, $p = 0.0001$) and (B, $p = 0.0002$).

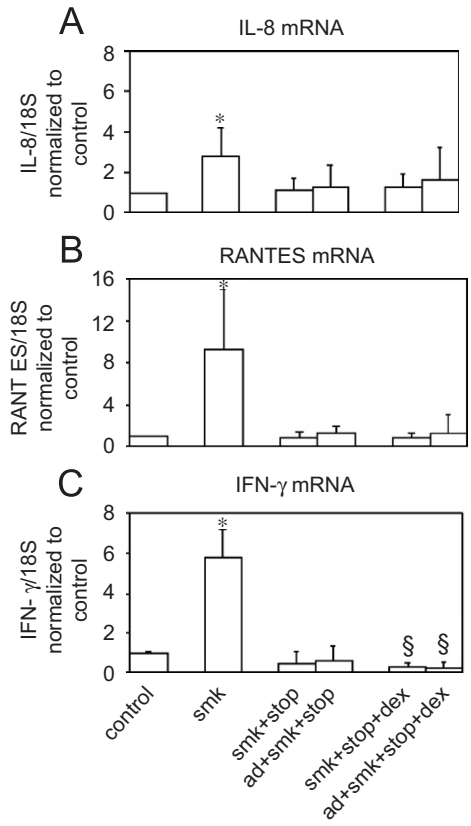


**Figure 4** Inflammatory mediator mRNA expression in lungs of guinea pigs. Results of quantitative real-time PCR of IL-8 (A) and RANTES (B) mRNA normalized to the corresponding 18S rRNA expression and semi-quantitative PCR of IFN-$\gamma$ mRNA (C) normalized to 18S rRNA. Each treatment group is normalized to its respective control where IL-8/18S ratios are $0.0016 \pm 0.0002$ and $0.005 \pm 0.007$ (A), RANTES/18S ratios are $0.0015 \pm 0.001$ and $0.0057 \pm 0.001$ (B) and IFN-$\gamma$/18S ratios are $0.00003 \pm 0.00007$ and $0.0052 \pm 0.001$ (C) for phase 1 and 2 controls, respectively. See Figure 1 for treatment group designations. *Main effect of smoke exposure (A, $p = 0.037$), (B, $p = 0.004$) and (C, $p = 0.009$); §Main effect of steroid treatment compared to control (C, $p = 0.001$).

initiated by smoking cessation. Latent adenoviral infection was also associated with lung inflammation but did not induce steroid resistance.

Smoking cessation was also associated with a decrease in CD4 cells, a reduction of neutrophil infiltration to control levels and a partial reversal of macrophage infiltration toward control values. These findings contrast sharply with those observed in several human studies[2,4,5] where an active inflammatory process persists in the lung long after stopping smoking. We suspect that the distinction between human and guinea pig is related to a relative difference in level of smoke exposure that can be achieved in rodents compared to that received by humans with a long term smoking habit. Retamales et al.[2] reported that mild human emphysema resulted in a reduced SA/volume ratio with preservation of the SA indicating that the earliest lesions are related to an increase in lung volume whereas severe human disease was associated with a marked destruction of the lung's SA. Although exposure to cigarette smoke has produced the equivalent of mild disease in several animal models we are unaware of any animal experiment that has produced the level of lung destruction observed in human emphysema.

In this study, steroid treatment reduced the accumulated volume of infiltrating neutrophils below that achieved by stopping smoking in both the lung parenchyma and airway wall with or without latent adenoviral infection while studies of induced sputum in COPD indicate that steroid treatment is not effective in reducing neutrophils.[8–10] Steroids also reduced eosinophil infiltration into airway walls whether latent adenoviral infection was present or not. These results indicate that latent adenoviral infection does not confer steroid resistance to cigarette smoke-induced lung inflammation in guinea pigs even though it can block the anti-inflammatory effects of steroids in allergen-induced eosinophil infiltration by preventing the down regulation of eotaxin production by steroids.[18] Latent adenovirus, however, can contribute to the inflammatory process, as shown in this study, by increasing airway wall neutrophils and CD8 cells. Decreases in inflammatory cells as a consequence of stopping smoking discussed above may relate to similar reversals currently demonstrated in the smoke-induced increased expression of inflammatory mediators. Decreased expression of IL-8, a cytokine that is chemotactic for neutrophils,[19] when smoke exposure was stopped, is likely the reason why less neutrophils are found in the parenchyma at this time. Interestingly, IL-8 levels in sputum of COPD patients who had quit smoking for at least 1 year did not decrease.[20] However, IL-8 levels did not change following steroid treatment as also observed by Keatings et al.[21] after 2 weeks of oral steroid in COPD patients. Other factors that are important in neutrophil migration include the expression of adhesion molecules ICAM-1. Studies have reported an increase of ICAM-1 in lungs of COPD patients[22,23] suggesting that neutrophils and the endothelium have been activated, and this is supported by the observation that circulating neutrophils from COPD patients have increased surface expression of ICAM-1 receptor CD11b compared to controls.[24] Steroid are known to down regulate the expression of CD11b on neutrophils[25] suggesting that decrease of neutrophil count following steroid treatment could be associated with a decrease of their recruitment in the lung.

Similarly, decreased RANTES, a chemokine attracting monocytes/macrophages and T-cells,[26] could account for decreased CD4 T-cells as well as the partial reversal of the smoke-induced macrophage infiltration. The latter is supported by accumulation of BAL macrophages after tracheal instillation of RANTES in guinea pigs.[27] RANTES is upregulated in stable COPD but this increase is more important during acute exacerbations frequently associated with bronchial eosinophilia.[28] Our current result is the first report showing that cigarette smoke can upregulate RANTES expression in the absence of eosinophilic infiltration.[12] Galkina et al.[29] found that pulmonary RANTES expression contributes to the active CD8 T-cell transmigration into the normal interstitium through a pertussis toxin-sensitive (PTX-sensitive) signaling mechanism that is not dependent on inflammation. In this study, RANTES expression is not associated with CD8 modulation. Surprisingly, RANTES was resistant to steroid treatment in our model whereas when it is associated with an eosinophilc infiltration, RANTES expression is reduced by steroid in an allergic lung inflammation model.[18]

There is increasing evidence that COPD has a type 1 T-cell cytokine profile with an increase in IFN-$\gamma$ expression.[30–34] Interestingly, Wang et al.[35] used a mouse model with an inducible IFN-$\gamma$ gene to show that the over-expression of IFN-$\gamma$ can cause emphysema and an alteration in the pulmonary protease/antiprotease balance. The relationship between IFN-$\gamma$ and inflammatory cells mainly focuses on lymphocytes. As this is the signature cytokine for the $T_H1$ subset of CD4 lymphocytes,[36] its decrease with smoking cessation may be reflective of similar decreases in CD4 cells. On the other hand, IFN-$\gamma$ can activate macrophages to indirectly increase chemotaxis of specific T-cells.[37] Also, of the mediators studied, only IFN-$\gamma$ was reduced by steroid treatment. The inhibition of transcription and synthesis of IFN-$\gamma$ by corticosteroids was demonstrated in both in vivo and in vitro studies, using both resting T-cells as well as effector T-cell clones.[38,39] Indirectly, corticosteroids affect T-cell by inhibiting macrophage IL-12, a cytokine that is extremely potent in enhancing type 1 immune responses by inducing the lytic function of cytotoxic CD8 T-cells. IL-12 also enhances the production of IFN-$\gamma$ and IL-2 by T-cells. The reduced production of IL-12 by corticosteroid-treated macrophages resulted in a decreased ability to induce IFN-$\gamma$ by T-lymphocytes.[40] IL-12 expression is increased in smokers and COPD patients and inhaled steroid decreased the IL-12 levels with dose dependency in stable COPD.[41] So while IFN-$\gamma$ expression is returned to control levels by smoking cessation, corticosteroids could reduce IFN-$\gamma$ expression even further either directly by affecting T-lymphocytes or indirectly by inhibiting IL-12 expression by macrophages.

Our results indicate that smoking cessation increased the SA of the lung suggesting that lung tissue damaged by smoke undergoes some levels of repair in this animal model. Increases in SA that occur during lung development results from a septation process that adds alveoli to alveolar ducts. Although this process is thought to be complete at birth in guinea pigs,[42] our data raise the possibility that it might be reawakened during the repair processes that occur following the cessation of smoking. In an attempt to support this repair process, we analyzed the expression of both proliferating cell nuclear antigen, a cell proliferation marker, and TGF-$\beta$1 but results were inconclusive (data not presented), most likely because we had to rely on an antibody specific for the human form of these proteins, for lack of guinea pig specific ones. The mechanism of tissue destruction by smoke exposure has been studied in guinea pigs where smoke-induced increased collagenase[43] levels, as well as attenuation of tobacco smoke-induced emphysema by a broad spectrum MMP inhibitor,[44] have been reported. The mechanism of tissue repair after smoking cessation in this model, however, requires further investigation in order to support our preliminary, but nonetheless novel, evidence that smoking cessation results in a repair process.

We conclude that smoking cessation diminishes cigarette smoke-induced lung inflammation and initiates tissue repair. Latent adenovirus contributes to the inflammatory process without inducing steroid resistance. The addition of steroid therapy further reduces IFN-$\gamma$ expression and the infiltration of neutrophils and eosinophils. Most importantly the repair process associated with stopping smoking includes an increase in the SA that may be associated with an awakening of the septation process that increases alveolar number and SA during lung growth.

## Acknowledgments

Authors would like to thank Dean English, W. Mark Elliott, Stuart Green, Zara Ahmed, Karen Groden, Reese Rounds, Kevin Quinlan, Amrit Samra, Soraya Utokarpach, Fanny Chu and the late Dianne Minshall for their technical support. We also thank Dr. Teizo Yoshimura for the generous gift of guinea pig IL-8 cDNA, and Dr. Mizuno Satoko and Dr. Yasuo Kashiwasaki from Sumitomo Pharmaceutical Co. Ltd. for their collaboration and the gift of guinea pig IFN-$\gamma$ cDNA. This work was supported by grants from the Canadian Institutes for Health Research, AstraZeneca Canada Ltd. and GSK Canada Inc.

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